

SERVA Collagenase NB 8 for isolation of rat islets

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Introduction

Beta-O2 Technologies Ltd., is a biomedical company developing a proprietary implantable bioartificial pancreas (BAP). The device is designed as a treatment for Insulin-Dependent Diabetes Mellitus - Type I diabetes, and for a subgroup of Type II diabetes. The BAP uniquely facilitates continuous oxygenation of insulin producing Islets of Langerhans by means of photosynthesizing algae. The system is contained in an immune-protected environment and is designed as to be implanted subcutaneously. The Islets of Langerhans in the BAP sense glucose levels and secrete necessary levels of insulin and glucagon as to maintain normal blood glucose levels. The company is finalizing long term transplantation experiments (>1 month) in a rat model and will start a new line of trials in a monkey model in the near future.

For rat islets which are needed for development of a BAP, high and constant quality and functionality are indispensable. A prerequisite therefore is the application of high quality collagenase for islet isolation. Previously Roche Liberase RI was used for routine isolation of rat islets. SERVAs expertise convinced Beta-O2 PI to test the Collagenase NB 8 as an alternative collagenase blend.

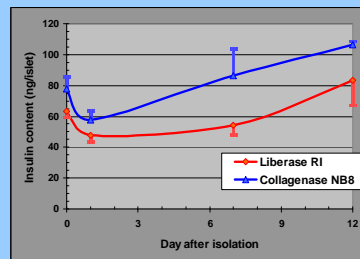
Methods

Islet isolations of Lewis male rat were performed with Collagenase NB 8 and Liberase RI. For Collagenase NB 8 best results were achieved with digestion time of 18 min by 15 PZ-U/pancreas. Yield, islet volume, insulin content and Glucose Stimulated Insulin Release (GSIR) were assessed and results compared with those of isolations performed with Liberase RI using standardized conditions which were established previously (9 PZ-U/ pancreas, 20 min). Function of islets isolated with Collagenase NB 8 was determined *in vivo* after transplantation of 1100 islets into diabetic rats.

Results

	Yield (islets/rat)	Number of isolations	Number of rats
Collagenase NB 8	705 ± 56.3	26	206
Liberase RI	623 ± 83.6	19	196

Number of islets were counted on day of isolation

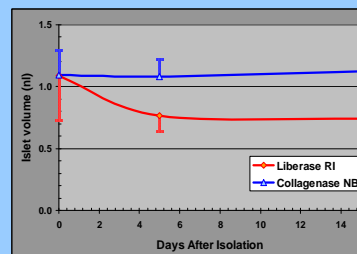


Insulin content of isolated islets was measured at day 0 or after 1, 7 or 12 days in suspension culture (density = 600 islets/90mm non-tissue culture Petri dish). Collagenase NB 8: n=9
Liberase RI: n=22

At any time of measurement the content of islet's insulin is higher for islets isolated with Collagenase NB 8 than for islets isolated with Liberase RI.

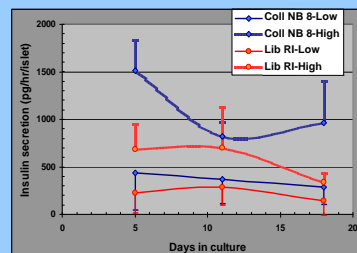
Conclusion

High yield of rat islets with high quality and functionality were achieved using Collagenase NB 8 for rat isolation. Reduced volume loss was shown for islets isolated with Collagenase NB 8 in comparison to islets isolated with Liberase RI which might indicate lower damage made to the cells on the surface of the islets by Collagenase NB 8. Good functionality of the islets was proven by GSIR which was higher for Collagenase NB 8 than for Liberase RI. For Collagenase NB 8 sustained islet graft function was also verified by *in vivo* assay. Rat islets isolated with Collagenase NB 8 exhibit best qualities which is indispensable for use as model in Beta-O2's application.



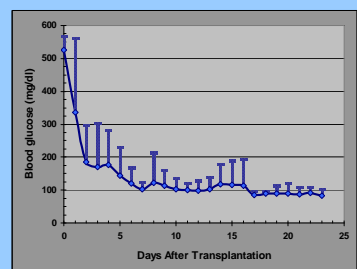
Islet volume (nl)- calculated from measured islet diameter of >100 islets. Collagenase NB 8: n=4
Liberase RI: n=9

Islets isolated with Collagenase NB 8 show reduced volume loss as compared to islets isolated with Liberase RI.



Islets (50/assay) were cultured in humidified CO₂ incubator in Krebs Ringer Buffer (KRB) with no glucose for a period of 60 min, then transferred to KRB containing 2.8 mM glucose (Low) for additional period of 60 min. Medium was harvested for insulin determination and glucose level increased to 16.7 mM (High). Insulin was measured by the Merckodia Insulin-ELISA system. Collagenase NB 8: n= 4
Liberase RI: n=11

Islets isolated with Collagenase NB 8 show higher GSIR and stimulation index at both Day 5 and Day 18 than islets isolated with Liberase RI.



Rats were made diabetic following IV injection of 85mg/kg BW of Streptozotocin. When non-fasting blood glucose reached >550mg/dl, a dose of 1100 islets, isolated with Collagenase NB 8, was transplanted under the right kidney capsule. Diabetes status was monitored twice daily by measuring non-fasting blood glucose. Results are mean ± SD. n=5 rats

After transplantation of islets into diabetic rats blood glucose level decreased rapidly and stayed at low level.

Data were kindly provided by



and are presented by

